PCR microarray probe circulating detection type biological chip (English Translation of CN1248702A)

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Abstract

The invented PCR microarray probe circulating detection type biochip relates to a new scheme of PCR microarray probe hybrid detection type biochip, specially it is a new type biochip which adopts gas or liquid reciprocating flow mode and multi-temperature zone PCR gene selective amplification and combines with solid-phase microprobe array technology to make gene-based diagnosis. It is characterized by that PCR circulation process and probe hybrid detection are designed in a PCR microarray probe chip, and the four microreactors of denaturation, annealing, elongation and microarray probe hybridization of PCR are formed into a reciprocating or encircling circulating system, and the temperatures of denaturation, annealing, elongation and hybridization in the circulating system are independently and thermostatically controlled. Several PCR microarray probe type chip systems can be integrated on the same chip.

PCR microarray probe circulating detection type biological chip

The invented PCR microarray probe circulating detection type biochip relates to a new scheme of PCR microarray probe hybrid detection type biochip. Specially, it is a new type biochip which adopts gas or liquid reciprocating flow mode and multi-temperature zone PCR gene selective amplification and combines with solid-phase microprobe array technology to make gene diagnosis.

Biological chip (biochip) is a kind of micro-analysis unit and system constructed on the solid chip surface by surface precision-machined technologies and super-molecule self-assembly technologies. One biochip system can include many units with different functions. One such example is a micro-fluidic biochip system integrated by biological sample pre-treatment unit, genetic materials extracting unit, specific gene fragment amplification unit, biological probe array unit and capillary electrophoresis unit, which is able to screen or detect compounds, proteins, nucleic acids, cells or other biological components quickly, precisely and in parallel. DNA chip with high density DNA probes is the most important biochip, which is able to analyze large number of genes quickly to facilitate scientists in getting and analyzing biological information.

Biochips are very useful tools in the fields of biological detection, medical analysis, drug discovery and gene sequence analysis. With the development of Human Genome Project (HGP), nucleic acids sequences and proteins sequences, structural data increase exponentially. The most challenging task next century is, after the finish of HGP, that is post-genome era, how we apply such large number of biological information to serve human society and revolutionize traditional medicine and therapy. Medicine nowadays is transforming from "the second period medicine on system, organ, tissue, cell level" to " the third period medicine on genomic, DNA—RNA—protein—protein and nucleic acid interaction and interaction with environment level". Such kind of genomic diagnosis and therapy based on molecule level will have the potential to make clear of disease mechanism even including cancer and cure them. The revolution in biology and medicine needs tools to detect and analyze genomic sequences first. HGP depends on whether scientists can sequence and analyze genome quickly and precisely. Traditional sequencing methods include chemical reactions, gel electrophoresis etc., which require complex and time-consuming steps, and are hard to integrate into portable instruments for quick sequencing. Applying micro-electronics and micro-mechanism technologies, biochip technologies integrate many discrete analytical processes such as sample preparation, chemical reactions and detection into chips. Biochip technologies will revolutionize disease diagnostics, drug discovery, food and environment etc. in the next century and provide powerful tools for collecting and analyzing biological information.

PCR (polymerase chain reaction) is a kind of gene amplification method in vitro. After 25-35 rounds, theoretically 10^6 copies will be amplified from one single

template. PCR technology had been widely used in research and clinical diagnostics for many years. Because of high false positive results, the use of PCR in clinical diagnostics was prohibited by Chinese National Health Department in June 1998. The false positive results were generally caused by: (1) false PCR amplification by unsteady factors of many experimental conditions (design and selection of primers, component of reaction solution, reaction time, temperature, cycle etc.); (2) Electrophoresis detection after PCR can only read results by fragment lengths not the sequences; and (3) PCR and electrophoresis are two discrete operations, and contamination can happen easily during the complex operations.

In recent years, biochip is an active frontier worldwide and develops quickly. Certain companies from US came into biochip field and produced biochips integrated PCR and DNA array together. There was a PCR micro-reaction chamber in these chip system. By controlling temperature circulation, gene amplification was done in the micro-reaction chamber. Then PCR products was sent to hybridization chamber to hybridize with solid-phase microarray probes and detected. Affymetrix designed its PCR micro-reaction chamber as a micro-fluidic channel. Most chips for research and clinical diagnostics are one or several independent devices. One single chip integrating PCR and chip detection together has been reported. PCR was run in one micro-reaction chamber. Precise temperature control (including elevating temperature and reducing temperature) to the same part of the device was needed. It was hard to monitor and analyze PCR results in real time. Even more, temperature setting needed time, the whole analysis time was extended.

The invented PCR microarray probe circulating detection type biochip is a new type biochip which adopts gas or liquid reciprocating flow mode and multi-temperature zone PCR gene selective amplification and combines with solid-phase microprobe array technology to make gene diagnosis. This design can simplify operations, shorten PCR time, elevate reaction efficiency, avoiding contacts with environment. Four micro-reaction chambers of denaturation, annealing, elongation and microarray probe hybridization of PCR are formed into a reciprocating or encircling circulating system, and the temperatures of denaturation, annealing, elongation and hybridization in the circulating system are independently and thermostatically controlled to avoid errors in temperature setting repeatedly in PCR or temperature controlling.

In the invented PCR microarray probe circulating detection type biochip, PCR circulating unit and probe hybridization unit are integrated in one PCR microarray probe chip. Four micro-reaction chambers of denaturation, annealing, elongation and microarray probe hybridization of PCR are formed into a reciprocating or encircling circulating system. It is easy to monitor PCR efficiency in every round by such design. The temperatures of denaturation, annealing, elongation and hybridization in the circulating system are independently and thermostatically controlled. Several PCR microarray probe type chip systems can be integrated on the same chip. Micro-reaction chambers for denaturation, annealing and elongation can be fabricated

in any types, such as micro-channels, micro-chambers etc. Microarray probes can be synthesized in situ or fabricated by arraying as low density or high density, mono-function or multi-function arrays. Micro-reaction chambers for annealing and elongation can be integrated in one chamber. After completion of the reaction in PCR microarray circulating detection type biochip, hybridization signals should be detected.

Our invention gives a new scheme of controlling PCR reaction solution circulating in different temperature zones to amplify. The invention combines PCR technology and DNA microarray technology into one integrated biochip system. Microarrays in the system can be high-density or low-density, fabricated by synthesis in situ or arraying, mono-function or multi-function. The design of our invention can simplify operations, reduce PCR reaction time, improve reaction products, avoid contamination, and read detection results by hybridization not by traditional electrophoresis. Because hybridization detection is sequence-specific, it can avoid false positive results, which is difficult for the traditional electrophoresis methods to avoid. It is more important that the design to integrate denaturation, annealing, elongation operations and microarray probe chip together and allocate denaturation, annealing, elongation and hybridization operations into four different constant temperature zones can avoid errors by setting temperatures repeatedly and controlling. Because of combining circulating PCR amplification technology and hybridization detection technology, sizes of each group chip can reduce greatly. It is possible to integrate several dozens or several hundreds chip units into one chip board to detect biological samples simultaneously. It is convenient to monitor reaction efficiency of every round in real time. It is possible to get linear PCR results and analyze quantitatively in real time. The device provides for quick, precise, automatic and clean operations.

The invented PCR microarray probe circulating detection type biochip has several detailed designs. We will give detailed explanations as follow with the help of figures.

Figure 1 is the sketch of invented PCR microarray probe circulating detection type biochip.

Figure 2 is one of invented PCR microarray probe circulating detection type biochip.

Figure 3 is view from the left of invented PCR microarray probe circulating detection type biochip in figure 2.

Figure 4 is another invented PCR microarray probe circulating detection type biochip.

Figure 5 is another invented PCR microarray probe circulating detection type biochip.

According to the figures, PCR microarray probe circulating detection type biochip integrates PCR amplification and probe hybridization. The four micro-reaction

chambers of denaturation, annealing, elongation and microarray probe hybridization of PCR are formed into a reciprocating or encircling circulating system, and the temperatures of denaturation, annealing, elongation and hybridization in the circulating system are independently and thermostatically controlled. Several PCR microarray probe type chip systems can be integrated on the same chip. Micro-reaction chambers for denaturation, annealing and elongation can be fabricated in any types, such as micro-channels, micro-chambers etc. Microarray probes can be synthesized in situ or fabricated by arraying as low density or high density, mono-function or multi-function arrays. Micro-reaction chambers for annealing and elongation can be integrated in one chamber. After the finish of the reaction in PCR microarray circulating detection type biochip, hybridization signals should be detected.

According to figure 1, in the invented PCR microarray probe circulating detection type biochip, denaturation is carried out in T1 micro-fluidic reaction chamber, annealing and elongation are carried out in micro-reaction chambers T2 and T3 respectively, and T4 is microarray probes chamber. Microarray probes can be synthesized in situ or fabricated by arraying as low density or high density, mono-function or multi-function arrays. V1 and V2 are T-connection valves. One PCR microarray probe circulating detection type biochip chip-1 includes units above (T1, T2, T3, T4, V1 and V2) and one PCR microarray probe circulating detection type biochip system for parallel samples analysis is a multi-chip board with some chip-1 structures.

Figure 2 and 3 gives one example of the invented PCR microarray probe circulating detection type biochip. Figure 2 is the main view of the invented PCR microarray probe circulating detection type biochip and figure 3 is the view from the left. There are micro-channels 1, 2, 3, 4, ..., n fabricated in substrate A. Each micro-channel connects with near micro-channels by micro-pores at the two end of the channel. There are four temperature zones T1, T2, T3 and T4 in the substrate A. These four temperature zones have different temperatures corresponding to temperatures for denaturation, annealing, elongation and hybridization. There are microarray probes in every micro-channel in T4 zone. And there are four channels fabricated in the rear of substrate A and these channels are perpendicular to micro-channels mentioned above. The micro-reaction chambers for denaturation, annealing and elongation can be fabricated in any types, such as micro-channels, micro-chambers. There are two small iron blocks in every micro-channel. And the blocks inosculate well with the micro-channel and can slide freely in the micro-channel. The surface of iron blocks are covered with a layer of hydrophobic organic polymer thin membrane. PCR reaction solution can be injected into the space between the two iron blocks. A transparent thin membrane is applied to cover the micro-channels and seal the solution into the space between blocks. Samples a, b, c, d, ..., n are injected into the PCR reaction solutions by puncturing covered membrane. After the solutions are mixed well, PCR reactions begin. Blocks and solutions between them can be moved

along the micro-channel to different temperature zones by magnet. The solutions are denatured in T1 zone first, then solutions are moved to T2 zone to anneal, then to T3 zone to elongate; after elongation, solutions are moved to T1 to begin the next round circulation or moved to T4 zone to hybridize first then to T1 zone to begin the next round circulation. Such circulation can be repeated till all the results are collected.

Figure 4 gives another example of the invented PCR microarray probe circulating detection type biochip. Comparing to biochip in figure 2 and 3, the invented biochip in figure 4 uses gases F1 and F2 but not magnet to move PCR reaction solutions 1, 2, 3, 4, ..., n circulating in the micro-channels. The other structures are the same as those in figures 2 and 3.

Figure 5 gives another example of the invented PCR microarray probe circulating detection type biochip. Micro-channels 1, 2, ..., n 's ends do not connect each other. There are other external channels connecting to both ends of micro-channels above. This design can avoid contamination and keep the micro-channels under one same pressure balanced condition to facilitate magnet N moving iron blocks and solution between them. The other structures and designs are the same as those in figure 2 and 3.

Example 1 (linear quantitative analysis): usage of HBV PCR microarray probe circulating detection type biochip (Figure 1). PCR reaction solution containing DNA template after simple pre-treatment is first flowed into T1 from F1, in which the DNA template is denatured for 45 seconds at 94°C, then flowed into T2 and annealed for 60 seconds at 45°C , then flowed into T3 and elongated for 2 minutes at 72°C, and at last enters into T4 along the route 1 through V1 and hybridizes with microarray probes to value efficiency of PCR reaction. If needed, the solution can enter into T1 along route 3 through V2 to begin the next PCR and hybridization round. After 30 rounds, the solution is guided out from F2 through V2 and hybridization signals are detected.

Example 2 (quantitative analysis): usage of HBV PCR microarray probe circulating detection type biochip (Figure 1). Patient blood sample (after pre-treatment) and PCR reaction solution is mixed well then flowed into T1 from F1. The operating program is as follows: T1 (94°C, 45 seconds)→T2 (45°C, 60 seconds)→T3 (72°C, 2 minutes)→V1→route 2→T1, for 30 rounds. After reaction, the solution is added into T4 from route 1 through V1 to hybridize with microarray probes. Liquid flows out from output F2 after hybridization. Hybridization signals are detected.

Example 3 (linear quantitative analysis): usage of multi-function multiple hepatitis viruses PCR microarray probe circulating detection type biochip (Figures 2, 3). One group patient blood samples after simple pre-treatment are first injected into PCR reaction solutions between iron blocks in micro-channels 1, 2, 3, ..., n; then a glass plate is covered on the chip to seal the solutions between iron blocks tightly and the solutions are mixed gently. The solutions move to T1 zone by magnet N under the

control of controller C5 and are denatured for 45 seconds at 94°C, then moved to T2 zone and annealed for 60 seconds at 45°C, then moved to T3 zone and elongated for 2 minutes at 72°C, at last enter into T4 zone and hybridize with microarray probes to value efficiency of PCR reaction. If needed, the solutions enter into T1 zone again to begin the next PCR and hybridization round. After 30 rounds, the hybridization results can be read by detecting hybridization signals and efficiencies of every round can be obtained by real time monitoring.

Example 4 (quantitative analysis): using high-density HBV PCR microarray probe circulating detection type biochip detecting SNP (Figure 4). One group patient blood samples 1, 2, 3, ..., n after simple pre-treatment are first injected into PCR reaction solutions between iron blocks in micro-channels 1, 2, 3, ..., n; then a glass plate is covered on the chip to seal the solutions between iron blocks tightly and the solutions are mixed gently. The solutions are moved to T1 zone by magnet N and denatured for 45 seconds at 94°C, then moved to T2 zone and annealed for 60 seconds at 45°C, then moved to T3 zone and elongated for 2 minutes at 72°C, then enter into T1 zone again to begin the next PCR round. After 30 rounds, the solutions are moved to T4 zone to hybridize with microarray probes. The hybridization results can be read by detecting hybridization signals.

Examples 5 and 6 are similar to examples 3 and 4. The only difference is that the moving of solutions in micro-channels is controlled by pressures on the ends of channels (Figure 4).

Examples 7 and 8 are similar to examples 3 and 4. The only difference is that the chip's structure is as shown in Figure 5.

In the Figures, small black block shows the micro-iron block and shadow shows the probe zone.

Claims:

1. A PCR microarray probe circulating detection type biochip, characterized in that (1) PCR amplification and probe hybridization are integrated into one PCR microarray probe chip; (2) The four micro-reaction chambers of denaturation, annealing, elongation and microarray probe hybridization of PCR are formed into a reciprocating or encircling circulating system and it is convenient to monitor reaction efficiencies in every round; (3) The temperatures of denaturation, annealing, elongation and hybridization in the circulating system are independently and thermostatically controlled; (4) Several PCR microarray probe type chip systems can be integrated on the same chip to become a PCR microarray probe chip system for multi-sample analysis.

2. The PCR microarray probe circulating detection type biochip of claim 1, wherein the micro-reaction chambers for denaturation, annealing and elongation can be fabricated in any types, such as micro-channels and micro-chambers.

3. The PCR microarray probe circulating detection type biochip of claim 1, wherein microarray probes can be synthesized in situ or fabricated by arraying as low density or high density, mono-functional or multi-functional arrays.

4. The PCR microarray probe circulating detection type biochip of claim 1, wherein the micro-reaction chambers for annealing and elongation can be integrated in one chamber.